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13. ABSTRACT

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Chemical structure studies have now established that the structural variation in Hb Ta-Li is located in the β chain at Position β-83 where a cysteinyl residue replaces the glycine group normally present at that location. In the propositus the relative amounts of Hb Ta-Li and Hb A_0, as determined by column chromatographic separations, were 40:60, respectively.

Hb Ta-Li is the second human hemoglobin variant known to involve the replacement of a normal constituent amino acid residue by a cysteinyl group. Previously Hb Pôrto Alegre, found in a Caucasian family in Brazil by other workers, was reported to be β_9 Ser→Cys. In both variants the change occurs at a position on the outside molecular surface which allows polymerization of the molecule by disulfide bonding. In spite of this unusual property both Hb's Ta-Li and Pôrto Alegre appear to function normally and cause no noticeable anemia in the bearer.

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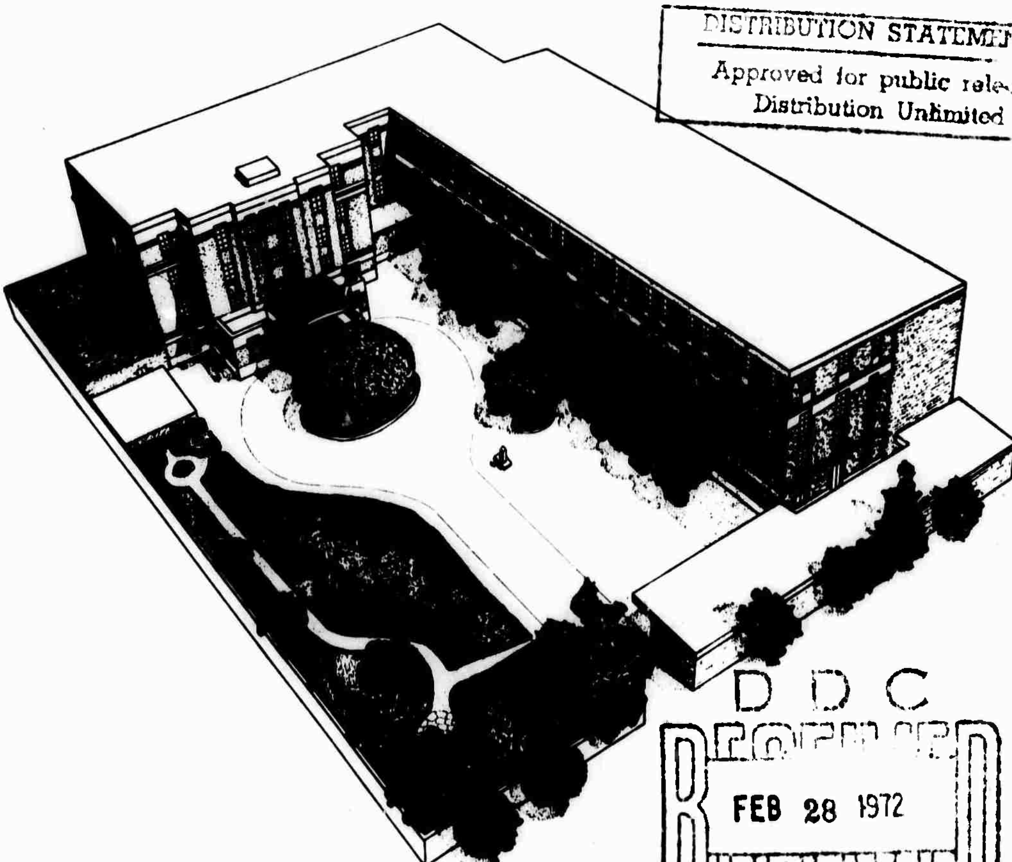

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HEMOGLOBIN Ta-Li: β83 Gly→Cys*

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(Received April 22nd, 1971)

SUMMARY

Hb Ta-Li was discovered in 1970 in one male Chinese subject during our continuing survey for hemoglobin variants among Chinese school children. The subject was born in Ta-Li, Taiwan and was of Taiwanese (Fukienese) ancestry. The brother and mother of the subject also were found to have the Hb Ta-Li; all three are heterozygotes.

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INTRODUCTION

Approx. 150 000 apparently normal Chinese subjects living in Taiwan have been screened for hemoglobin variants by starch-gel electrophoresis during the past several years (R. Q. BLACKWELL AND J. T.-H. HUANG, unpublished results). Among that number approx. 70 individuals were found to have singly slow variants characteristic

* The opinions and assertions contained herein are those of the authors and are not to be construed as official or reflecting the views of the U.S. Navy Department or the U.S. Naval Service at large.

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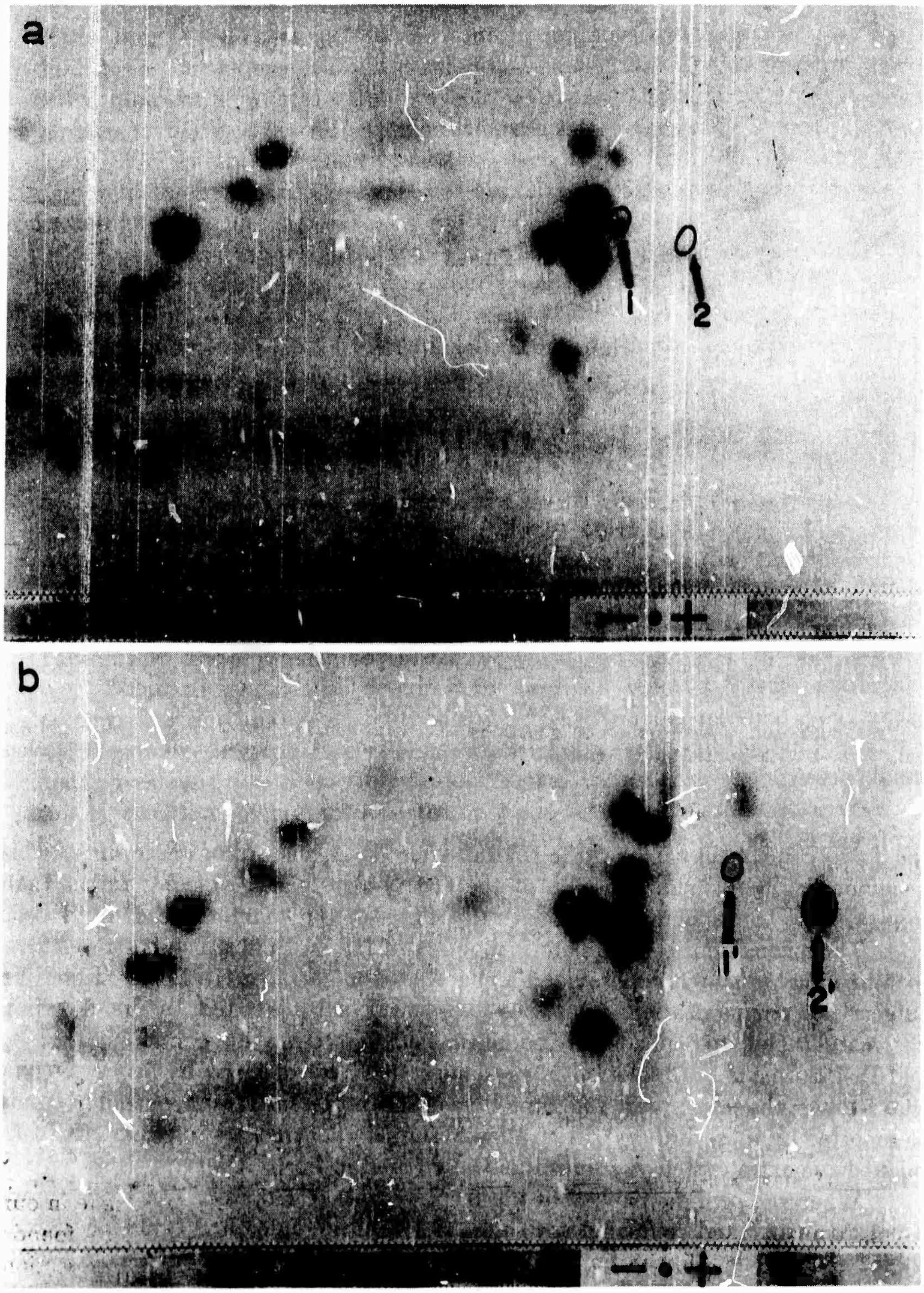

Fig. 2. Peptide maps of the core peptides. (a) The peptide map of the Hb A_0 core. (b) The peptide map of Hb Ta-Li core. Differences between the two maps are evident at Positions 1 and 2 on both maps. Positions 1 and 2 on (a) (from Hb A_0) were found by amino acid analyses to be Peptides βT10-11 and βT10, respectively. Positions 1′ and 2′ on (b) are the corresponding peptides β^{TT}10-11 and β^{TT}10 from Hb Ta-Li. The greater anodal mobility of the latter pair of peptides results from the presence in each of one additional cysteic acid residue.

of the G- or D-type hemoglobins and approx. 40 others had doubly slow variants of the E-type hemoglobins. Only one individual among the 150 000 subjects screened had a different slow hemoglobin variant which was characterized by an electrophoretic mobility in starch-gel intermediate between those of the singly slow and doubly slow variants. The new variant has been named Hb Ta-Li. Up to the present time only the index case and some of his relatives have been found to have this variant.

Structural analyses now have shown that the structural anomaly in Hb Ta-Li involves the replacement of a glycyl group by cysteinyl at the β-83 or βEF7 position; therefore Hb Ta-Li can be represented as $\alpha_2\beta_2^{83\mathrm{Gly}\rightarrow\mathrm{Cys}}$. As discussed below some of the characteristics of Hb Ta-Li resemble those of Hb Pôrto Alegre[1,2] which also has, in its β chains, an extra cysteinyl group.

MATERIALS AND METHODS

The Hb Ta-Li variant was found, along with Hb A_0, in the blood of a 17-year-old Taiwanese male born in the town of Ta-Li*, Taiwan. Blood samples also were obtained from the brother and sister of the index case as well as their mother. Starch-gel electrophoresis indicated that the index case, his brother and mother all have the hemoglobin variant in addition to normal Hb A_0. Both parents were born in Taiwan and are of Fukienese ancestry.

Standard methods as described in previous reports[3-6] were used in the present structural analyses. Dinitrophenyl derivatives for N-terminal group analyses were made by the procedure of SANGER AND THOMPSON[7] and the DNP-amino acid derivatives were identified by two-dimensional chromatography using polyamide thin-layer chromatography supported on polyester film as described by WANG AND WANG[8]. Dansyl derivatives were prepared as described by GRAY[9] and degradation of dansylated peptides carried out by his method[10,11]. The dansyl-amino acids were identified with polyamide thin-layer chromatography by the procedure of WOODS AND WANG[12]. Starch gel[13] and cellogel[14] electrophoresis were used for screening as well as for more detailed study of the Hb Ta-Li fractions.

RESULTS AND DISCUSSION

Hemolysates from both the index case and his brother, each containing Hb Ta-Li as well as Hb A_0, were studied by starch-gel electrophoresis[13] at pH 8.9 and by cellogel electrophoresis[14] at pH 8.8. The results in starch gel are illustrated in Fig. 1; the mobility of the Hb Ta-Li component in the starch gel was between that of Hb E and Hb G. By contrast in cellogel there was virtually no difference between the mobilities of Hb's Ta-Li and A_0. Attempted separation of the two components by starch block electrophoresis[15] with veronal buffer at pH 8.6 was unsuccessful; no band separation was achieved.

When the hemolysate from the index case was subjected to column chromatography on DEAE-Sephadex A-50-120 (ref. 16) using 0.05 M Tris–HCl gradient buffers from pH 7.8 to pH 7.0, the two components A_0 and Ta-Li were separated. However,

* Pronounced, "Dah-Lee".

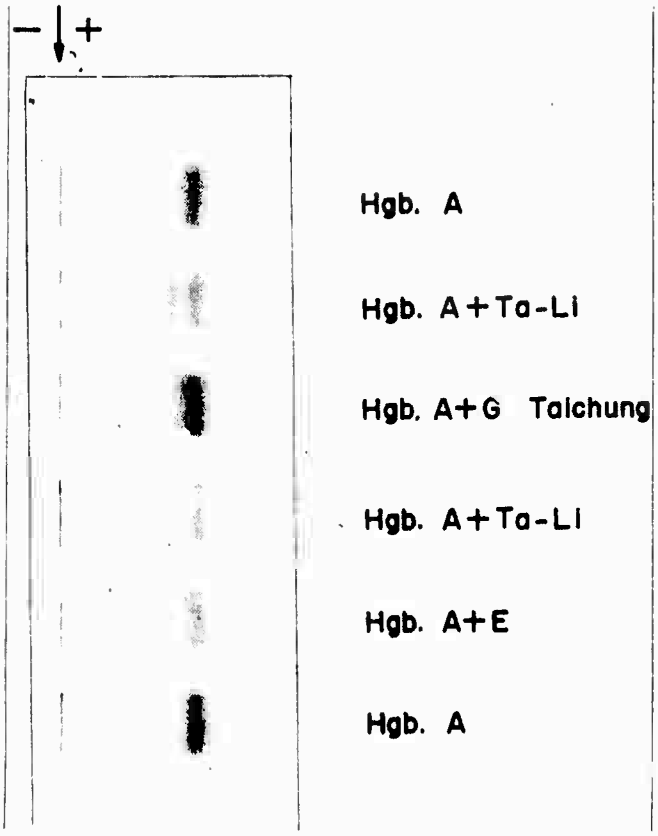

Fig. 1. Comparison of electrophoretic migration of several hemoglobins in starch gel at pH 8.9 using Tris–EDTA–borate buffer. The Hb Ta-Li polymer is seen to migrate between Hb E and Hb G Taichung.

the Ta-Li component came off the column after Hb A_0 instead of ahead of A_0. This behavior has been found in some of the fast, J-type hemoglobins[5,17] rather than slow, G-type hemoglobins which generally elute off the column ahead[6] of Hb A_0.

The inconsistent electrophoretic mobility of Hb Ta-Li in which it showed slow mobility only in starch gel provided a clue that it might, like Hb Pôrto Alegre[1], be subject to polymerization. This possibility was substantiated by gel-filtration chromatography, on Sephadex G-100, of the hemolysate mixture containing Hb's A_0 and Ta-Li in 0.2 M NaCl solution as was done with Hb Pôrto Alegre by BONAVENTURA AND RIGGS[2]; the results were the same type of separation into two hemoglobin bands as reported by those authors[2]. From those results it appeared possible that Hb Ta-Li might have extra cysteinyl groups like those found in Pôrto Alegre.

When the peptide map[18,19] of the tryptic digest of Hb Ta-Li was made in our usual manner[3-6] and compared with that of Hb A no difference could be found; these results made it likely that the structural change was located in the core section of the molecule. Accordingly, 15 to 30 mg quantities of the insoluble core residues remaining from regular tryptic digestion of both Hb's A_0 and Ta-Li were washed with buffer at pH 6.4, dissolved in 0.1 M HCl, reprecipitated with acetone and washed with acetone as previously described[20,21]. Following this the core material was treated with performic acid[22] at 0° for 2 h after which the mixture was diluted with ice-cold water to stop oxidation; the sample was lyophilized and again subjected

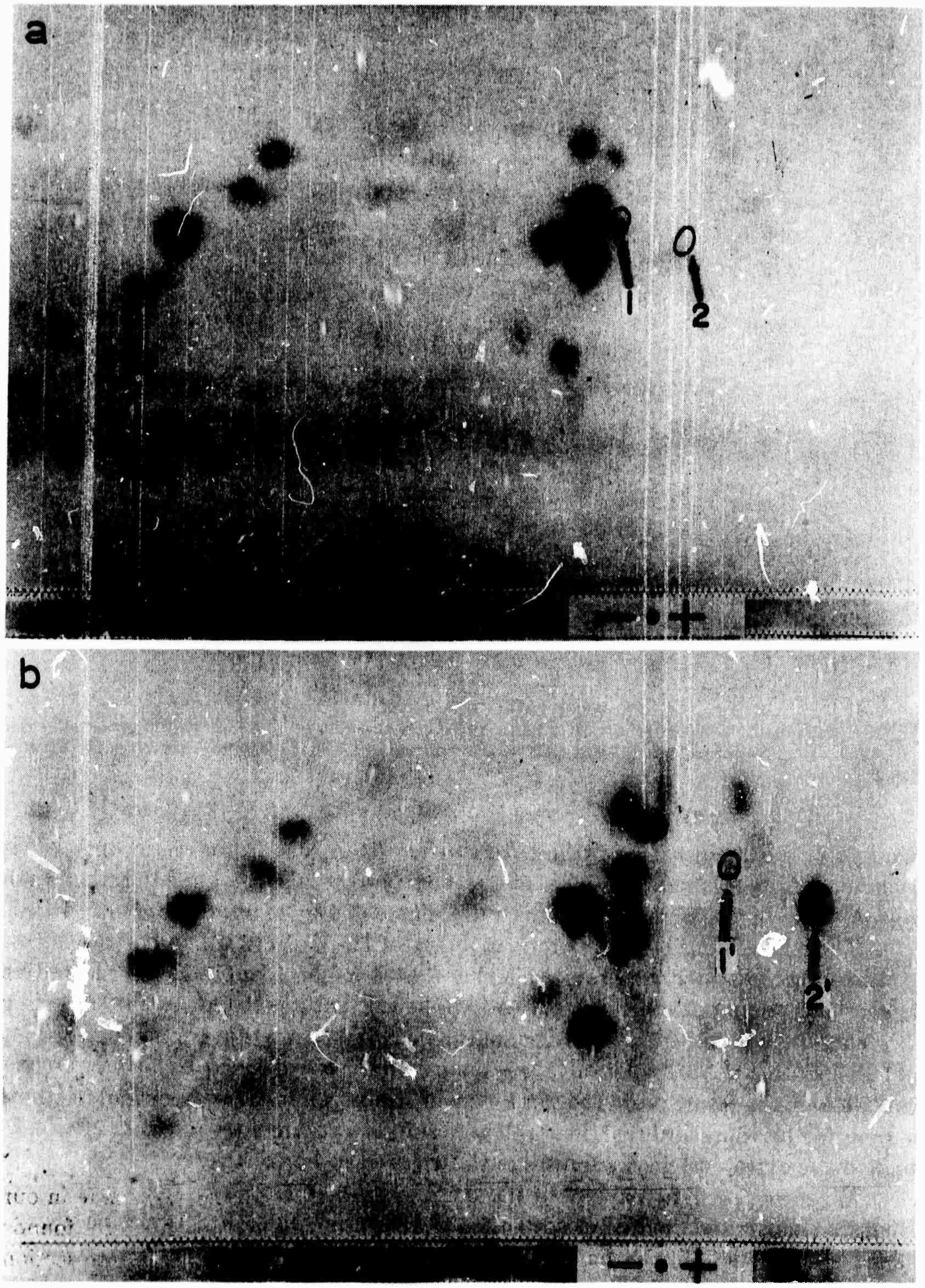

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TABLE I

AMINO ACID COMPOSITIONS OF THE NORMAL βT10 AND βT10-11 PEPTIDES FROM Hb A COMPARED WITH THOSE OF THE CORRESPONDING ABNORMAL β^{T}T10 AND β^{T}T10-11 PEPTIDES FROM Hb Ta-Li

Amino acid residue	*Expected*			*Observed*							
	βT10 Molar ratio	*βT11 Molar ratio*	*βT10-11 combined Molar ratio*	*βT10*		*βT10-11*		*β^T T10*		*β^T T10-11*	
				nmoles	*Molar ratio*	*nmoles*	*Molar ratio*	*nmoles*	*Molar ratio*	*nmoles*	*Molar ratio*
Asp	1	2*	3*	20	1.0	220	2.7	62	0.97	215	2.9
Glu	1	1	2	23	1.1	170	2.1	66	1.0	160	2.2
Ser	1		1	20	1.0	81	1.0	64	1.0	76	1.0
Thr	2		2	38	1.9	140	1.7	115	1.8	145	2.0
Gly	1		1	17	0.85	68	0.85	5	0.08	5	0.07
Ala	1		1	21	1.0	100	1.2	62	0.97	86	1.2
Val		1	1			80	1.0			74	1.0
Leu	2	1	3	39	1.9	220	2.7	130	2.0	245	3.3
Phe	1	1	2	21	1.0	170	2.1	65	1.0	160	2.2
Pro		1	1			60	0.75			66	0.89
Cys**	1		1	24	1.2	77	0.96	120	1.9	130	1.8
His	1	1	2	18	0.90	150	1.9	68	1.1	145	2.0
Lys	1		1	20	1.0	86	1.1	63	0.98	76	1.0
Arg		1	1			79	1.0			67	0.91

* Including one Asn residue.
** Determined as cysteic acid.

–Gly–Thr–Phe–Ala–Thr–Leu–Ser–Glu–Leu–His–Cys–Asp–Lys–
83 85 90 93 95
βT10

–Leu–His–Val–Asp–Pro–Glu–Asn–Phe–Arg–
96 100 104
βT11

Fig. 3. Peptides βT10 and βT11. The cysteine group normally present at Position β-93 occurs in the present βT10 peptide as a cysteic acid group because of performic acid oxidation treatment of the "core" section of the hemoglobin molecule prior to tryptic hydrolysis. The presence of the cysteic acid residue at Position β-93 along with the aspartic acid residue at Position β-94 partially inhibited tryptic digestion of the peptide bond between the lysyl group at β-95 and the leucyl group at β-96. Hence part of the βT10 and βT11 peptides were found together as Peptide βT10-11. Peptide β^{T}T10 differs from βT10 (and β^{T}T10-11 from βT10-11) only in the replacement of glycine at Position β-83 by another cysteic acid group.

to tryptic digestion in the regular manner for 2.5 h with an approximate enzyme–substrate weight ratio of 1:50 in one run and 1:30 in another.

The resulting peptide maps are illustrated in Fig. 2. Fig. 2a shows the map of the Hb A_0 core peptides and Fig. 2b illustrates the peptides found in the Hb Ta-Li core. The peptide spots on both maps appeared to be similar except for the pair marked 1 and 2 on Fig. 2a and 1′ and 2′ on Fig. 2b. Amino acid analyses were made on material eluted from those positions on the peptide maps. The amino acid compositions, given in Table I, indicated that peptides at Position 1 and Position 2 of the map from Hb A_0 were, respectively, βT10-11 and βT10. The corresponding amino acid compositions for the peptides at Positions 1′ and 2′ of the map from Hb Ta-Li were the same except for the replacement in each case of a glycyl residue by a cysteinyl

residue; therefore the peptides were designated β^{T}T10-11 and β^{T}T10, respectively. In this case all of the cysteinyl residues present were determined as cysteic acid because of the preliminary performic acid treatment to make the core material more susceptible to tryptic digestion by conversion of cysteinyl groups to cysteic acid groups. The conversion of the cysteinyl group at Position β-93 to cysteic acid in addition to the presence of the aspartyl group normally at Position β-94 interfered with the hydrolytic cleavage of the peptide bond between the lysyl group at Position β-95 and the leucyl group at Position β-96 and accounts for the presence of Peptides βT10-11 and β^{T}T10-11. This fact was confirmed when the amount of trypsin was increased to an approximate enzyme–substrate weight ratio of 1:15; the resulting peptide maps of both sets of core peptides (not illustrated) showed proportionally more βT10 and β^{T}T10 peptides and less βT10-11 and β^{T}T10-11 peptides than were present in the earlier peptide mixtures.

As shown in Fig. 3, the only glycine in Peptide βT10 is at the N-terminal, β-83, position. Therefore, if cysteine replaced glycine in Peptide βT10 it would occur at the β-83 position. The difference in the electrophoretic mobilities seen between the Peptides βT10 and β^{T}T10 and between Peptides βT10-11 and β^{T}T10-11 can be explained by the presence of one additional negatively charged cysteic acid residue in the Peptides β^{T}T10 and β^{T}T10-11 in place of the neutral glycyl groups in Peptides βT10 and βT10-11.

To verify the replacement of glycyl by cysteinyl at Position β-83, a sample of β^{T}T10-11 peptide was eluted from a peptide map with 1% trimethylamine solution and the N-terminal group tagged with dinitrophenol[7]. After acid hydrolysis of the dinitrophenyl-tagged peptide the mixture of amino acids and the dinitrophenyl-tagged amino acid from the N-terminal position of the peptide were subjected to two-dimensional thin-layer chromatography[8]. DNP-cysteic acid was identified as the dinitrophenyl-tagged amino acid arising from the N-terminal (β-83) position. Additional evidence for the presence of N-terminal glycine in Peptides βT10 and βT10-11 was the transient yellow color[23] noted for both peptides on the peptide map immediately after ninhydrin staining. By contrast the β^{T}T10 and β^{T}T10-11 peptides did not show the transient yellow color but developed the blue color directly.

Finally, degradation studies were made on Peptides βT10-11 and β^{T}T10-11 using GRAY's dansylation procedure[9-11]. In Peptide βT10 the sequence found as expected, beginning with the N-terminal position, was glycine, threonine, phenylalanine, and alanine; in Peptide β^{T}T10-11 the first three positions were cysteic acid, threonine, and phenylalanine. Therefore the N-terminal portion of the two peptides differed only in the initial, N-terminal positions. Taken together these data are considered adequate to establish the structural anomaly in Hb Ta-Li to be β83 Gly→Cys.

Hb Ta-Li is the second human hemoglobin variant to be reported in which a cysteinyl group replaces another amino acid residue. In Hb Pôrto Alegre[1], cysteinyl replaces seryl at the β-9 position[2]. Both Pôrto Alegre and Ta-Li were found primarily because of their slow mobilities in starch gel at alkaline pH values. In both hemoglobins the altered mobilities are not due to the usual reason of an altered charge but rather to altered size. The two hemoglobins can polymerize by the formation of intermolecular disulfide bonds; their polymerization is facilitated by the fact that the positions of the additional cysteine group, β-9 or βA6 and β-83 or βEF7, are

located on the external surface[24] of the molecule. The present results, however, offer no explanation as to why normal hemoglobin A_0 does not polymerize through its cysteinyl groups at β-93 (βF9) and β-112 (βG14) which also occupy external surface positions[24].

No detailed family studies have been made to determine the possible effects of Hb Ta-Li in the bearer, however, the original subject had no obvious complaints of anemia. His red blood cell count and hematocrit value were normal, $4.4 \cdot 10^6$ and 47%, respectively; the reticulocyte count was unremarkable, 0.4%.

Hb Ta-Li showed a slight heat instability by the method of KLEIHAUER AND GORDON (ref. 25 and private communication); the amount of precipitate was relatively smaller than that seen, for example, in Hb E and Hb K Kaoshiung (Hb New York)[26]. The relative amounts of Hb's Ta-Li and A_0 as measured by DEAE-Sephadex separations on two occasions were 38:62 and 41:59; from these values the proportion can be estimated to be approx. 40:60.

Further studies are required to determine whether appreciable levels of polymerization of Hb Ta-Li occur *in vivo*; however, it is considered likely that the amount of polymerization is quite low under normal physiological conditions.

No variants have been reported previously at the β-83 or βEF7 position. However at the corresponding position in the α chain, the αEF7 position or α-78, the change Asn→Lys has been reported in Hb Stanleyville II (ref. 27).

NOTE ADDED IN PROOF (Received June 17th, 1971)

Shortly after the present paper was submitted for publication two reports[29,30] appeared concerning Hb Rainier, β145 Tyr→Cys. There appears to be no tendency on the part of Hb Rainier to polymerize in contrast to that discussed above for Pôrto Alegre and Ta-Li. Instead, the new cysteinyl residue in Hb Rainier promotes the formation of an intrachain disulfide bond with the -SH group on the normally occurring cysteinyl residue at Position β-93, *i.e.* at the position adjoining the proximal histidinyl group at Position β-92. The formation of the intramolecular disulfide bonds in Hb Ranier produces marked effects on the performance of the molecule; it has an abnormally elevated resistance to alkaline denaturation, increased oxygen affinity and reduced alkaline Bohr effect. Clinically the molecular changes result in a chronic polycythemia; no such effect was seen in Hb Ta-Li.

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